

THE LIFE HISTORY AND MORPHOLOGY OF *MACRAVESTIBULUM*  
*EVERSUM* SP. NOV. (PRONOCEPHALIDAE, TREMATODA)

DORIS YIN-MING HSÜ

A dissertation submitted in partial fulfillment of the requirements  
for the degree of Doctor of Philosophy in the University of Michigan.

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LIFE HISTORY AND MORPHOLOGY OF *MACRAVESTIBULUM*  
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INTRODUCTION

Although the Family *Pronocephalidae* Looss was erected in 1902, and some of its members have been known since 1809, information concerning it relates chiefly to records and to morphology and taxonomy of various species. The investigation here reported is the second to deal with the life history of a member of the family. Because of the increasing emphasis placed during the past two decades upon the excretory system as a criterion of relationship among digenetic trematodes, particular attention has been paid to the development of this system, and much attention has been devoted to the development of the reproductive system.

HISTORICAL REVIEW

Looss (1899) erected the subfamily *Pronocephalinae* to include monostomes of marine turtles, which have a robust and somewhat elongated body, rounded anterior end, cephalic collar, truncate posterior end, and concave ventral side. In this subfamily he included three new genera: *Pronocephalus*, *Cricocephalus*, and *Pyelosomum*. To these he (1901) added four more new genera, namely, *Charaxicephalus*, *Adenogaster*, *Pleurogonius*, and *Glyphicephalus* and listed a number of species in marine turtles as belonging to them. Later, he (1902) elevated the subfamily *Pronocephalinae* to familial rank and included in the new family an eighth genus *Epibathra* created by him. The genera *Barisomum* Linton (1910), *Desmogonius* Stephens (1911), *Diaschistorchis* Johnston (1913), *Wilderia* Pratt (1914), *Synechorchis* Barker (1922),† *Hippocrepis* Travassos (1922), *Astrorchis* Poche (1926), *Opisthoporus* Fukui (1929), *Macravestigibulum* Mackin (1930), *Neopronocephalus* Mehra (1932) were later added to the family. Price (1931) presented a classification of the Family *Pronocephalidae* with three subfamilies, *Opisthoporinae*, *Charaxicephalinae* and *Pronocephalinae*. Mehra (1932) erected a new genus, *Neopronocephalus*, and a new subfamily, *Neopronocephalinae*, for two new species found in freshwater turtles. He recognizes three subfamilies, namely, *Pronocephalinae*, *Neopronocephalinae*, and *Charaxicephalinae*. A few months later, Mehra (1932a) combined his own earlier classification with that of Price (1931), accepted *Opisthoporinae*, and added a new subfamily, *Hippocrepinae*, thus

\* Contribution from the Department of Zoology, University of Michigan.

† According to Price (1931) the genera *Wilderia* and *Synechorchis* are synonymous with the genus *Diaschistorchis*.



giving a revised classification of *Pronocephalidae* with five subfamilies and sixteen genera. Within these genera thirty-two species were recognized of which four occur in fresh-water turtles, three in marine fish, one in aquatic rodents, and the rest in marine turtles. To these must now be added *Macravestibulum eversum* sp. nov. from a fresh-water turtle.

The first species of the family *Pronocephalidae* found in North America and the first to be found any where in fresh-water turtles was *Opisthoporus aspidonectes* (MacCallum). Ascribed erroneously by MacCallum (1917) to the genus *Paramphistomum*, Fukui (1929) accepted it as an amphistome and erected for it the genus *Opisthoporus* and the family *Opisthoporidae*. Stunkard (1930) correctly determined it to be a monostome of the family *Pronocephalidae*. He had for study new material collected from the fresh-water turtle, *Amyda spinifera*. The second member of the family obtained from fresh-water turtles is likewise from North America and was reported by Mackin (1930), under the name *Macravestibulum obtusicaudum*. Mehra (1932) described from some fresh-water turtles in India three species, one in the genus *Diaschistorchis*, and two in *Neopronocephalus*. The present species is the second belonging to the genus *Macravestibulum* Mackin and the third North American pronocephalid from fresh-water turtles.

#### ACKNOWLEDGMENTS

I wish to express my sincere appreciation to Professor George R. La Rue, under whom this research was conducted, for his interest, criticisms and suggestions concerning this work. I also wish to thank Professor Arthur E. Woodhead for his guidance of the research during Professor La Rue's absence, Dr. Henry Vander Schalie of the Museum of Zoology for identifying the snail host, Dr. Helen F. Price for friendly assistance, and Mr. Chishen Chen for assistance in the collection of snails and in devising an apparatus for this purpose.

#### MATERIAL AND TECHNIQUE

Redial and cercarial stages were obtained from the snail, *Goniobasis livescens* (Menke). For experimental final hosts several species of fresh-water turtles were used, viz. *Emys blandingii* (Blanding's turtle), *Chelydra serpentina* (snapping turtle), *Chrysemys bellii marginata* (western painted turtle), *Pseudemys elegans* (western painted terrapin), *Amyda spinifera* (soft-shell turtle), *Sternotherus odoratus* (musk-turtle), and *Graptemys geographica* (map-turtle). During the spring of 1933, the snail hosts were collected from Honey Creek west of Ann Arbor, Michigan. Of the 1288 snails there collected 0.23 per cent were infected. Since the summer of 1933 snails have been collected from the Huron River at Delhi Woods Park, about seven miles northwest of Ann Arbor. Of the 3707 snails collected from the river 2.65 per cent were infected, a higher percentage of infection than

in Honey Creek. Turtles are also more abundant in the river than in the creek. Most of the collections of snails were made during the spring, summer and early fall. Since the snails remain infected the entire year, they were sometimes collected during cold weather.

During warm weather the snails, when brought to the laboratory, were placed individually in small bottles of tap water, and observed daily for cercariae. At the approach of cold weather the cercariae stop emerging even in the laboratory and dissection of snails was necessary to obtain rediae and cercariae. Both living and stained specimens were studied. Rediae with developing cercariae were dissected from the gills of snails, and by teasing open the rediae with fine needles these embryos were set free. They were put in normal salt solution and studied alive. Some were fixed in Bouin's solution and stained in Mayer's paracarmine or haemalum for whole mounts; others were sectioned and stained either in Flemming's triple stain or in one part of Mayer's haemalum plus two parts of 2 per cent ammonia alum. Mature cercariae were studied alive and also from permanent whole mounts or stained sections. Some cercariae were preserved in hot 10 per cent formalin for measuring.

Metacercariae were removed by means of needles from cysts taken from the protected surface of the opercula of the snail hosts. The metacercariae were studied alive and as whole mounts stained in paracarmine.

Each turtle used in feeding experiments was forcibly fed with a number of cysts at intervals of a few days, and later was killed and examined. The intestines were removed, slit open and examined carefully under a binocular microscope. Sometimes the intestines were shaken or their contents were scraped into salt solution. After decanting, the parasites were easily picked out. The adults were studied alive and again after staining and mounting following fixation in Bouin's solution. Some were stained either in Mayer's haemalum or Delafield's haematoxylin for whole mounts, while others were sectioned in frontal or transverse planes and stained in Mayer's haemalum diluted with six parts of 2 per cent ammonia alum solution for six or seven hours. Reconstructions of the genital systems of the adults were made from camera lucida drawings.

*Macravestibulum eversum* sp. nov.

Specific Diagnosis: *Macravestibulum*. Adults small, thick and spatulate, opaque, light brown in color, 0.9–1.215 mm. long (average 1.032 mm.) by 0.36–0.495 mm. broad (average 0.447 mm.). Cephalic collar prominent, muscular, incomplete ventrally. Body bluntly rounded or truncate posteriorly, sides almost parallel, in fixed specimens convex dorsally, concave ventrally. Oral sucker small, ventral, subterminal, 0.094–0.114 by 0.094–0.11 mm. (average 0.09 by 0.093 mm.). Intestinal ceca unbranched, ending in posterior fourth of body. Excretory pore a large, terminal, transverse



slit surrounded by heavy muscles. Bladder in two parts: the vestibular part with two eversible lateral pouches with many folds and derived from ectoderm, the other Y-shaped and derived from mesoderm. Genital pore lateral, sinistral, intracecal or ventral to cecum, at first third of body length. Cirruspouch large, curved, oblique. Testes two, slightly asymmetrical, in posterior third of body. Ovary dorsal, dextral, anterior to right testis. Mehlis' gland median, partly covered by ovary. Vitellaria compact, follicular, anterior to testes, chiefly intracecal. Uterine coils transverse, intracecal. Metraterm large, muscular. Eggs small, colorless, with long filament at each pole. Cercariae in *Goniobasis livescens* (Menke); adult in map-turtle, *Graptemys geographica* (Le Sueur).

Description: *Macravestibulum eversum* is a small worm obtained from the upper third of the small intestine of the map-turtle, *Graptemys geographica* (Le Sueur). It is opaque, light-brown, and capable of a considerable degree of contraction and extension. Both anterior and posterior ends are strongly muscular (fig. 11). The former is furnished with heavy longitudinal and circular muscles of the body plus the muscular bands that form the collar, while the posterior end is provided with a strong muscular band that surrounds the large excretory pore. The cuticula is thin and smooth. Five specimens in permanent mounts measure 0.9–1.215 by 0.36–0.495 mm. (average 1.032 by 0.447 mm.).

Digestive system: The oral sucker is ventral and subterminal. The esophagus is thin-walled, long, narrow, and without a pharynx; it bifurcates at the anterior third of the body. The intestinal ceca are smooth and unbranched. They pass dorsally and almost laterally to testes, being overlapped by the latter, and curve toward the median line behind the testes where they end blindly.

Excretory system: The excretory pore is a large terminal opening surrounded by heavy muscles and leading into a vestibular cavity. The excretory bladder consists of two parts: a Y-shaped primary bladder derived from the original mesodermal excretory tubes of the young cercaria, and a secondary bladder with two eversible lateral chambers with cuticula-covered walls thrown into many folds. The primary bladder receives two main excretory trunks joined anteriorly and with a short, blind, median pouch behind the oral sucker (fig. 11). The detailed morphological account of this system will be discussed later in connection with its development.

Male reproductive organs: The testes, situated in the posterior third of the body, are slightly asymmetrical in position. The left testis lies slightly anterior and dorsal to the right, is spheroidal, smaller than the right, and measures 0.09–0.14 by 0.08–0.12 mm. (average 0.121 by 0.098 mm.). The right testis is large, ovoidal, oblique to the long axis of the body, and measures 0.12–0.18 by 0.072–0.12 mm. (average 0.15 by 0.096 mm.). Anterior to the uterine folds, and lying free in the parenchyma, is the long,

## EXPLANATION OF PLATES

## Abbreviations:

|            |                                        |
|------------|----------------------------------------|
| <i>bp</i>  | birth pore                             |
| <i>cp</i>  | cirruspouch                            |
| <i>ctd</i> | common vitelline duct                  |
| <i>ebp</i> | primary bladder                        |
| <i>ebs</i> | secondary bladder                      |
| <i>ep</i>  | excretory pore                         |
| <i>fcp</i> | fundament of cirruspouch               |
| <i>fmg</i> | fundament of Mehlis' gland             |
| <i>fmi</i> | fundament of metraterm                 |
| <i>fo</i>  | fundament of ovary                     |
| <i>ft</i>  | fundament of testis                    |
| <i>ftv</i> | fundament of vitellaria                |
| <i>gpr</i> | genital primordium                     |
| <i>lc</i>  | Laurer's canal                         |
| <i>lo</i>  | locomotor organ                        |
| <i>lp</i>  | lateral pouch of the secondary bladder |
| <i>mg</i>  | Mehliss' gland                         |
| <i>mt</i>  | metraterm                              |
| <i>oot</i> | oötype                                 |
| <i>ovd</i> | oviduct                                |
| <i>p</i>   | pore between two bladders              |
| <i>sc</i>  | somatic cell                           |
| <i>ut</i>  | uterus                                 |
| <i>vd</i>  | vas deferens                           |
| <i>ve</i>  | vasa efferentia                        |
| <i>vt</i>  | vitellaria                             |
| <i>vd</i>  | vitelline duct                         |
| <i>vtv</i> | vitelline reservoir                    |

## EXPLANATION OF PLATE I

Figures 1, 3, 5, 7, 8, 23-27 and 30-35 are free-hand drawings; all others were made with the aid of a camera lucida.

Unless otherwise stated, the value of the scale is 0.2 mm.

- FIG. 1. Redia containing second generation of rediae.  
 FIG. 2. Redia containing cercariae.  
 FIG. 3. Excretory system of redia.  
 FIG. 4. Locomotor organ of cercaria.  
 FIG. 5. Locomotor organ, much protruded, showing minute spines and two sets of muscles.  
 FIG. 6. Nervous system of cercaria.  
 FIG. 7. Posterior part of adult with lateral pouches of secondary bladder partly everted.  
 FIG. 8. Posterior part of adult with lateral pouches of secondary bladder fully everted.  
 FIG. 9. Reconstruction of female genital system in transverse plane, anterior view.  
 FIG. 10. Reconstruction of female genital system in frontal plane, ventral view.  
 FIG. 11. Adult *Macravestibulum eversum*, ventral view.  
 FIG. 12. Metacercaria, ventral view.  
 FIG. 13. Uterine egg from terminal portion of uterus.  
 FIG. 14. Normally emerged cercaria, ventral view.



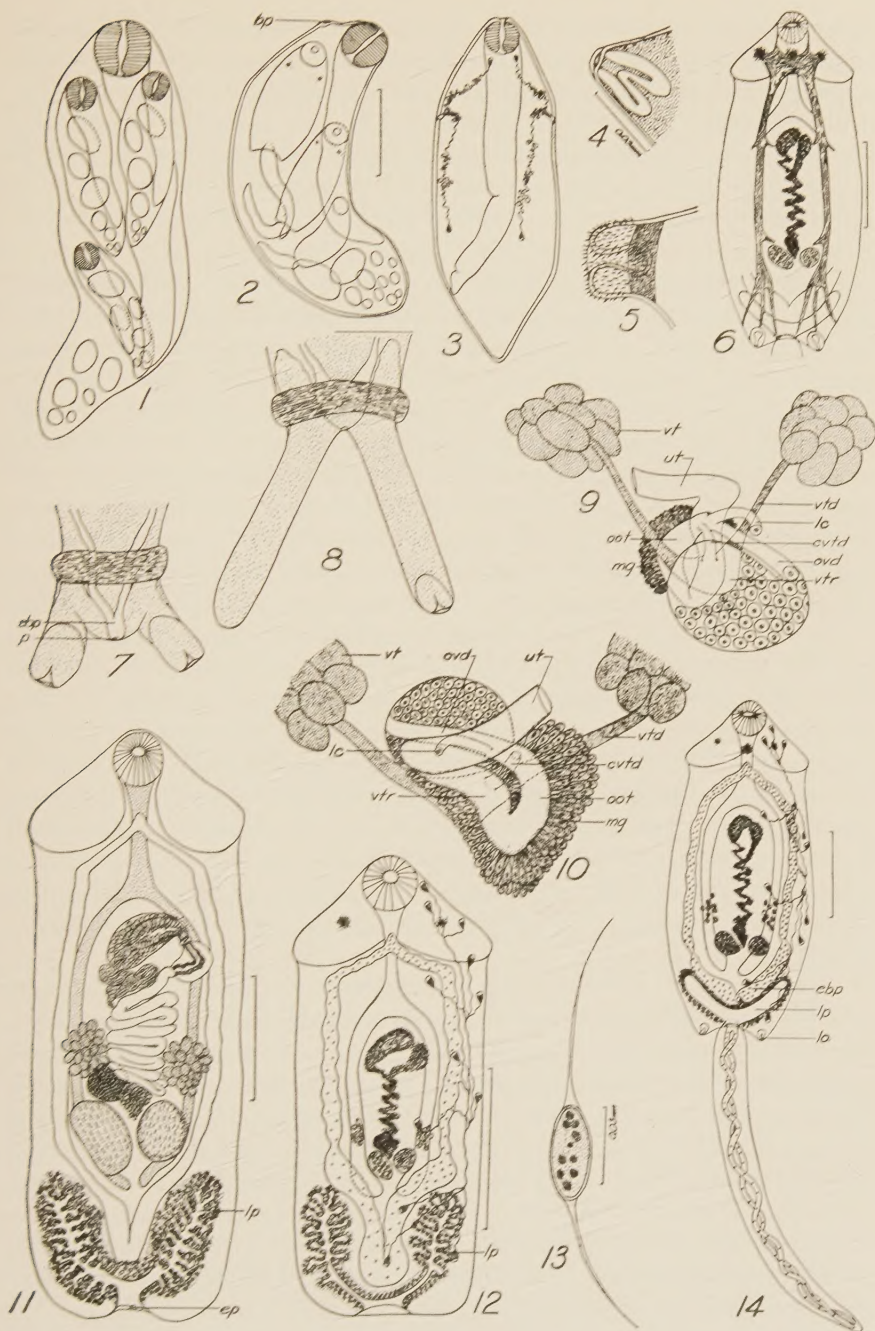


PLATE I

coiled seminal vesicle which opens into the cirruspouch where it widens and coils to join the pars prostatica. The cirruspouch is large and curved, almost transverse to the long axis of the body, and measuring 0.146–0.19 by 0.08–0.102 mm. (average 0.169 by 0.095 mm.). The pars prostatica is large and occupies most of the cirruspouch, while the cells of the prostate gland fill up the space between it and the wall of the cirruspouch. The cirrus is short and capable of being protruded only a short distance. The genital atrium is small.

Female reproductive organs: The ovary is globular, dorsal, slightly dextral, and anterior to the testes, and measures 0.09–0.093 mm. in diameter. In a contracted worm when viewed from the ventral side, it is more or less hidden by Mehlis' gland, testes, and vitellaria. Mehlis' gland is median, ventral to, and partly overlaps the ovary. The oviduct arises at the right margin and ventral side of the ovary, and curves ventrad and mesiad. At this point Laurer's canal emerges, passes a short distance ventrally and then dorsally to open near the posterior end of the ovary. The oviduct extends posteriorly and to the left in a ventral position and just before entering the oötype surrounded by Mehlis' gland, it receives the common vitelline duct. From the oötype the uterus continues forward as numerous transverse coils between the intestinal ceca. The metraterm is large, muscular, and measures 0.082–0.106 by 0.64–0.08 mm. (average 0.101 by 0.074 mm.). The vitellaria consist of two compact clusters of spherical follicles, situated anterior to the testes and ventral to the intestinal ceca, partly overlapping the latter. The vitelline ducts run diagonally posteriad to the region near the right margin of Mehlis' gland where their junction forms the vitelline reservoir from whose ventral side the common vitelline duct extends anteriad until it joins the oviduct (figs. 9 and 10). Twenty uterine eggs at the distal end of the uterus near the metraterm measure, exclusive of the polar filaments, 0.028–0.032 by 0.01–0.012 mm. (average 0.029 by 0.0108 mm.).

Discussion: *M. eversum* differs in some respects from *M. obtusicaudum* which is relatively larger, with "the anterior part of the body highly muscular, posterior part with only thin musculature." In *M. eversum* the posterior end, as well as the anterior end, is strongly muscular, since there is a heavy muscular band around the excretory pore. Aside from differences in the positions of Mehlis' gland and the vitellaria, they differ in the structure of the metraterm. In *M. obtusicaudum* this organ is short and narrow, while in *M. eversum* it is large, well-developed, muscular, and about two-thirds the size of the cirruspouch. The excretory system presents other points of difference. In *M. obtusicaudum* a long medium canal almost reaching the oral sucker extends anteriorly from the circuitous tubule, and the two excretory trunks send out a number of short lobate projections from the side walls in the region lateral and posterior to the testes, while in



*M. eversum* the blind anterior median excretory pouch is short, and the walls of the excretory trunks are smooth and unbranched. Other differences in the excretory system will be discussed later in connection with its development. All the above-mentioned dissimilarities furnish conclusive evidence that these forms are congeneric, but not conspecific.

#### LIFE HISTORY\*

During the spring of 1933 some snails, *Goniobasis livescens* (Menke), were collected from Honey Creek, a small stream west of Ann Arbor, and when brought to the laboratory, trioculate monostome cercariae with a prominent cephalic collar were found emerging from one of them. Since it was known that monostome cercariae encyst in the open, lettuce leaves and other plants were exposed to the cercariae, but no encystment took place on them, on the dish containing the snail, or on the shell of the snail. Later, in cracking open snails to study the young cercariae, a number of white, translucent, pearl-like cysts were found on the protected surface of the operculum. The cysts were opened, examined microscopically, and the metacercariae identified as those belonging to a pronocephalid species. Later, under the binocular microscope cercariae were observed creeping under the operculum and encysting there.

Fresh-water turtles were used as hosts for these metacercariae. Forty to fifty cysts were forcibly fed once a week for four weeks to a fresh-water turtle, *Pseudemys elegans*. Five weeks after the first feeding the turtle was killed, the intestines examined carefully, and thirty worms of various sizes and stages of development were obtained, none sexually mature.

Different species of turtles were used as experimental hosts, namely, two *Emys blandingii* (Blanding's turtle), three *Chelydra serpentina* (snapping turtle), eight *Chrysemys bellii marginata* (western painted turtle), six *Pseudemys elegans* (western painted terrapin), two *Amyda spinifera* (soft-shell turtle), four *Sternotherus odoratus* (musk-turtle), and six *Graptemys geographica* (map-turtle). Each turtle was forcibly fed several times at intervals of a few days with cysts wrapped in raw meat.

These experiments showed that *M. eversum* could live in *Chelydra serpentina*, *Chrysemys bellii marginata*, and *Pseudemys elegans* for a time without reaching maturity but finally died. In *Chrysemys bellii marginata* the worms lived somewhat longer, about six to seven weeks, without becoming mature, and then disappeared completely from the turtle. They could not

\* Since this paper was written Horsfall's brief observations on the life-history of *M. obtusicaudum* have appeared (Horsfall, 1935). Comparison of the two reports shows that the two species are distinct, yet they have much in common. It is also patent that Miss Hsü overlooked the paper by Horsfall (1930) on *Cercaria infracaudata* which the same author (1935) has shown to be the cercaria of *M. obtusicaudum*. I have taken the liberty of inserting from time to time references to, and discussions of, these two papers.—(George R. La Rue.)



establish themselves in *Emys blandingii*, *Amyda spinifera*, and *Sternotherus odoratus*. It was only in the map-turtle (*Gratemys geographica*) that they came to full development. Horsfall (1935) reported *M. obtusicaudum* as natural infections in *Pseudemys elegans*, *P. concinna*, *P. troosti*, *Gratemys geographica* and *G. psudogeographica*. She was unable to infect *Amyda spinifera*, *Sternotherus odoratus*, *Chelydra serpentina*, and *Chrysemys marginata*.

Since molluscs are the main diet of the map-turtle, six of these turtles were obtained for feeding experiments, three in September and three in October, 1933. They were forcibly fed with cysts wrapped in ground beef. Four of the turtles died within the first six weeks and worms were found in them. One turtle, killed and examined in December, 1933, i.e., 93 days after the first feeding, and 42 days after the last feeding, yielded 26 worms, none of which was sexually mature. The other turtle, fed with the same number of cysts, was killed in April, 1934, i.e., 221 days after the first feeding, and 172 days after the last feeding, but it was not infected with any of these worms.

Later, seventeen *Gratemys geographica* were obtained from Bruin Lake, about twenty-two miles northwest of Ann Arbor, where *Goniobasis* does not occur. These map-turtles were treated first with tetrachlorethylene to rid them of their parasites. About two weeks later they were put in an aquarium and allowed to feed spontaneously on snails known to harbor cysts. In spite of special effort and great care, nine of these turtles died before the parasites reached maturity. From three of the eight survivors, eighteen sexually mature adults were obtained. Seven to eight weeks were required for the parasites to reach maturity. Although many parasites were present, only a few matured at a time. Toward the latter part of the summer and during the fall no parasite becomes mature no matter how long it has been living in the host. Turtles examined during the fall of 1933 and in the fall of 1934 after a period of infection of seven to eight weeks, or longer, harbored many worms of which none was sexually mature. Failure to mature might be due to the physiological condition of the turtles, which instead of hibernating, were forced to remain active.

In June, 1934, a map-turtle was obtained from the Huron River near Delhi Woods Park where the snail hosts were generally secured. When examined, about two hundred pronoccephalids were found in its intestines, but only one was barely mature, and contained a few eggs in the uterus. In both naturally and experimentally infected turtles these parasites were mostly found in the upper part of the small intestine though a few were found in the lower part.

This life history can be briefly stated thus: Cercariae are produced in rediae in the snails, *Goniobasis livescens* (Menke), and emerge into the water. They soon encyst on the protected surface of the opercula of the

same species of snail, which is later eaten by the map-turtle (*Gratemys geographica*). In the stomach or upper intestine the parasites are released and attach themselves by the oral sucker to the intestinal wall, where they develop to maturity after seven to eight weeks.

#### REDIA

Numerous rediae and cercariae in various stages of development are found in the gills of infected snails, *Goniobasis livescens* (Menke). The mature redia is an elongated sac, narrow at the anterior, and wider at the posterior end which sometimes slopes down to a blunt point. The mouth is terminal and opens immediately into a more or less spherical, muscular pharynx. The intestine is voluminous, reaches almost to the posterior extremity of the body, and is filled with reddish orange food particles. The body wall is muscular and the redia has considerable power of extension and contraction. No locomotor appendages are present at any stage of development.

The excretory system of the redia is of the usual type with the flame cells and tubules of one side separate from the other. The anterior pair of flame cells lie just behind the pharynx and the posterior pair in the last third of the body.

A birth pore is adjacent to the pharynx (fig. 2). The body cavity is filled with developing cercariae, generally no more than two to four, the more well-developed embryos being toward the anterior part, while young germ-balls of various sizes occupy the posterior part of the cavity. Cercariae escape from the redia before development is complete. They remain in the tissues of the gills, increase in size, develop a median eye and more pigmentation before emerging from the snail.

Ten mature rediae, killed in hot 10 per cent formalin solution, measured 0.486–0.648 mm. by 0.306–0.369 mm. (average 0.535 by 0.345 mm.). The pharynx measured 0.06–0.096 mm. by 0.06–0.1 mm. (average 0.0726 by 0.0738 mm.) and the gut 0.369–0.45 mm. by 0.09–0.135 mm. (average 0.405 by 0.114 mm.).

Two generations of rediae occur since in the gills of a naturally infected snail there were found, together with rediae containing developing cercariae, a few rediae filled with young rediae (fig. 1). The sporocyst, however, has not been observed, since no experimental infections of snails with miracidia were made.

Redia producing monostome cercariae have been described by various investigators,\* but it seems that the only description of the excretory system of a monostome redia was given by Faust (1919a) who described

\* Horsfall (1930) found the redia of *M. obtusicaudum* (*Cercaria infracaudata*) in the livers of *Goniobasis livescens tricta*. The redia found by her has a much longer gut than this species and apparently no birth pore or the latter was overlooked (La Rue).

two flame-cells on each side of the redia producing *C. spatula*. My findings in regard to the excretory system in the present redia agree with Faust's. A birth pore has been described for monostome rediae only by Szidat (1930), who mentioned the presence of a birth pore in *C. ephemera* Nitzsch. The number of cercariae in the redia of *M. eversum* is small, never exceeding four. Sewell (1922) in the redia of *C. indicæ* XI found only one or two cercariae. Lagrange (1919) stated that the redia of a monostome cercaria contained only two to four cercariae. Faust (1918) in *C. aurita*, (1924), *C. hemispheroides* and (1930) *C. yenchingensis* reported that there were only a few cercariae at a time in the redia. Wesenberg-Lund (1934) wrote that there were but few cercariae in the redia of *C. ephemera* and rarely more than two to four in the redia of *C. imbricata*. It seems, therefore, that the small number of cercariae contained in the redia is a common feature, perhaps the rule, in monostome life histories. Wesenberg-Lund (1934) wrote: "It is a common feature of all true monostomes that the development of the cercariae is not completed in the redia but in the tissues of the snail." But according to Faust (1917), the cercariae usually remain until maturity in the redia of *C. pellucida*. In *M. eversum* development of the cercaria is completed in the host tissues, confirming Wesenberg-Lund's statement. Furthermore, the presence of only two eye-spots in the cercariae inside the redia of the present species is similar to the findings in *C. monostomi* described by Wesenberg-Lund (1934). The latter further stated that although it has been doubted whether in monostomes there is normally a number of generations of rediae or a single generation of rediae producing cercariae, Lühe (1909) had reported rediae producing rediae, and he himself (1934) had seen this in *C. imbricata*. In the present species, rediae producing rediae were observed only once. Most monostome cercariae reported in the literature have their site of infection in the liver and a few in the interhepatic lymph sinuses of the snail hosts. This species, however, infects the gills of the snail and this appears to be the first report of such a locus for monostome cercariae and rediae. The infection is carried over winter, since infected snails are found throughout this season.

Wesenberg-Lund (1934) stated, "No sporocyst stage and development of the cercariae outside the rediae, would seem fairly probable. Omitting the sporocyst stages the Monostomes have not the same power of enormous germ production as most of the other Trematoda. For this purpose they only possess the redia stage." While it is possible that the sporocyst stages are omitted in the monostomes, non-observance of sporocysts does not prove their non-existence. Furthermore, since Joyeux (1922) found the sporocyst of *Notocotylus attenuatus* Rud. producing rediae, it is certain that a sporocyst may be present in monostomes. Failure to observe sporocysts may be accounted for on the following grounds: the number of the sporocysts in any snail is small, since a single sporocyst is derived from a



miracidium, and it is probable that multiple infections are not common, since snails may develop immunity (Nolf and Cort, 1933); the sporocyst is comparatively small and readily overlooked; the life of the sporocyst is relatively short; in some trematodes, e.g., schistosomes (Price 1931), the mother sporocyst is located in the head and not in the liver of the snail where the infection is usually sought; snails which do not yield cercariae are rarely dissected with great care, and it is these only which are likely to yield the sporocyst; the miracidia of some species penetrate only into young snails (Ameel, 1934), and the sporocyst is gone before the snail is half grown. Thus the probability of obtaining the mother sporocyst in natural infections is small. Furthermore, no monostome life history has been completed in which a miracidium has been described, and no sporocyst has been obtained experimentally except by Joyeux (1922).

#### CERCARIA

The cercariae of *M. eversum* are large, muscular, capable of considerable extension and contraction, and are elongate-ovoid or spatulate when fully extended. The tail, equaling the length of the body, is also capable of considerable extension and contraction. Measurements in millimeters of twenty-five cercariae fixed in hot 10 per cent formalin are as follows: body, 0.495–0.855 mm. (average 0.605 mm.) long by 0.144–0.333 mm. (average 0.21 mm.) wide; tail, 0.459–0.702 mm. (average 0.579 mm.) long by 0.063–0.099 mm. (average 0.084 mm.) wide near the base; oral sucker, 0.054–0.072 by 0.045–0.054 mm. (average 0.061 by 0.05 mm.).

The mature cercaria has three eye-spots with lenses, and a prominent collar just behind the eye-spots. This collar, which resembles that of an echinostome cercaria except that it is without spines, encircles the anterior end from dorsal to ventral side where it is interrupted by a gap at the median line. Around the three eye-spots a heavy pigmentation of brown granules extends outward and forward around the oral sucker and backward along the dorsal surface in two lines which serve as the superficial indices of the underlying nerve trunks. The lateral eye-spots are dark brown and conical in shape, and each has a lens fitted into a cup-shaped mass of pigment. The median eye-spot is lighter brown than the lateral ones, and its lens is fitted into a cup of more diffused pigmented granules. The two lateral eye-spots develop in the young embryo, but the median eye-spot does not develop until the cercaria has escaped from the redia and is in the tissue of the snail.

The whole body is opaque and packed with cystogenous cells filled with a rod-like granular substance. The cuticula of both body and tail is smooth with the exception of the cavities of postero-lateral locomotor organs which are spinose. These locomotor organs, which take the place of the ventral sucker during locomotion, are located at each postero-lateral angle of the

body (fig. 6). They originate early in the young embryo after the formation of the tail, as infoldings of the tissues taking the cuticula with them (fig. 24). As the invagination advances a central funnel-shaped cavity is formed which opens to the exterior. Meanwhile, surrounding this funnel-shaped cavity, a group of large glandular cells are differentiated (figs. 25, 26, and 27). Later, the bottom of the cavity begins to evaginate, finally forming a fold which when completed gives the cavity the form of a "Y" (fig. 4). Minute spines develop in the cuticula and cover the entire inner surface of the invaginated part. Two sets of muscles, longitudinal and circular, are developed around the organ (fig. 5). The large glandular cells surrounding the cavity secrete a sticky substance into the lumen. When the circular muscles contract, the pocket is evaginated, forming a projection and carrying with it the glandular secretion. Both spines and secretion aid the locomotor pockets in securing a strong hold on the substratum. By means of the oral sucker and the locomotor pockets whose form undergoes great variation by the alternate action of circular and longitudinal muscles, the animal creeps like a measuring worm. While crawling, the body is capable of great alteration in form. When swimming the body is contracted to oval shape, while the tail actively vibrates and thus drives the cercaria forward through the water. Periods of activity alternate with periods of rest. These cercariae always swim toward the most brightly illuminated part of the dish. After periods of swimming the cercaria settles to the bottom or on the side of the container, and begins to creep.

The digestive system is of the usual type with bifurcated ceca; mouth ventral, slightly subterminal; oral sucker muscular; esophagus long, narrow, and without a pharynx. The intestinal bifurcation is at the anterior fourth of the body, and the ceca run in a straight course, slightly dorsal and medial to the excretory trunks, to the posterior part of the body, where they end blindly behind the testes (fig. 14).

The genital system is well-developed with its parts already differentiated. Ovary, testes, and Mehlis' gland lie near one another in the posterior third of the body, the ovary slightly dextral and dorsal to Mehlis' gland which is median, while the testes are lateral and posterior to ovary and Mehlis' gland. The vitellaria in front of the testes and ventral to the ceca consist of a few scattered follicles with the vitelline ducts passing posteriad and mesiad to the Mehlis' gland. The vasa efferentia cross the vitelline ducts, and in front of the ovary they join the vas deferens which

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#### EXPLANATION OF PLATE II

FIGS. 15-22 and 29. Developmental stages of reproductive system from germ-ball to mature cercaria.

FIGS. 23-28. Drawings showing processes in formation of secondary bladder.

FIGS. 30-35. Developmental stages of excretory system from germ-ball to the stage in which formation of primary bladder and excretory pore is completed.

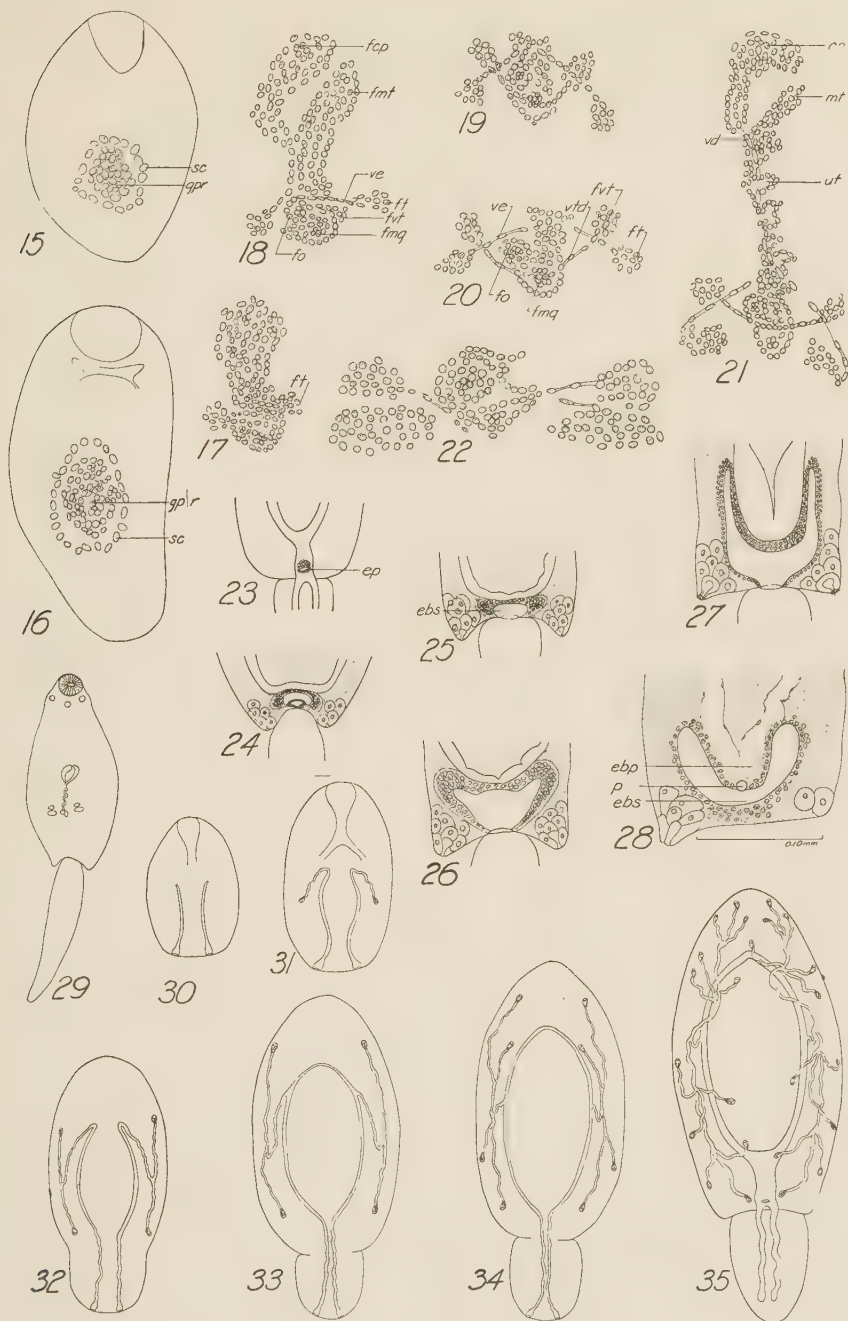


PLATE II



extends anteriad, dorsal to the uterus. At its anterior end the vas deferens expands into a cirruspouch which lies obliquely transverse to the long axis at the anterior third of the body. Passing anteriorly along the median line, ventral to the vas deferens, is the long, slender, somewhat coiled uterus which enlarges just lateral to the cirruspouch to form the metraterm (fig. 14).

The excretory system (fig. 14) consists of two trunks joined anteriorly and discharging posteriorly into the urinary bladder which is composed of two parts, a primary and a secondary bladder whose development will be discussed later. The excretory pore opens dorsally at the junction of the tail and body. In the tail two excretory tubes extend from the bladder to the posterior end of the tail where they terminate blindly. The two main excretory trunks, extending forward and laterally from the urinary bladder, and forming a tubular circuit just behind the median eye, are filled with excretory concretions. Near the middle of each main excretory trunk a short branch connects the lateral collecting tubules and capillaries with their flame-cells to the main trunk (fig. 14). There are twelve pairs of flame-cells whose pattern is expressed by the formula  $2[(3+3)+(3+3)]$ .

The nervous system (fig. 6) consists of an anterior ganglionic mass, situated at the level of the eye-spots, and two main nerve trunks running posteriorly from the ganglionic mass along the dorsal side. Two nerves arise from the ganglionic mass, and extend forward to the oral sucker. The lateral eye-spots are innervated by two short optic nerves which extend laterally and dorsally from the brain center, while a single nerve extending anteriorly and dorsally innervates the median eye. At the level of the cirruspouch and metraterm, each dorsal trunk gives off two lateral nerves, one extending mesiad, the other toward the body wall; farther back another pair of nerves extends toward the sex glands. Each trunk divides into three branches at the posterior end, one extending to the excretory pore, one to the locomotor organ, and the other ending laterally on the body wall, anterior to the locomotor organ. Detailed discussion of the development of excretory and genital systems will be given later.

The cercariae encyst on the protected surface of the operculum of uninfected and infected *Goniobasis*, rarely on the shell. Apparently the greatest emergence of cercariae occurs between one and four P.M. After swimming in the water for a time, these cercariae begin to creep on the side of the dish where the snails generally crawl along. They then creep under the operculum of the snail, i.e., between the operculum and the tissue, and encyst there. The process of encystment is carried out in the usual manner and is completed in four or five minutes, but it sometimes requires as much as eight minutes. A cyst in side view is hemispherical, the attached surface being flat. Its diameter is 0.277 mm. The encysted stage occurs most abundantly during the summer months. About 80 to

90 per cent of the snails carry cysts, the number of cysts varying from one to seventeen or eighteen.

Discussion: Although many monostome cercariae have been described only one,\* viz. *C. indicae* XI Sewell (1922), of all the descriptions examined by me, can be definitely assigned to the *Pronocephalidae*. Faust (1917a) classified all monostome cercariae into two groups, the "Binoculate" including those with two eye-spots and the "Trioculate" with three. Sewell (1922) divided the monostome cercariae into six groups, of which his "Ephemera" group includes those forms in Faust's "Trioculate" group plus *C. monostomi* v. Linst., *C. ephemera* Lebour, and *C. indicae* XI Sewell. These cercariae, with the exception of *C. indicae* XI which is a pronocephalid cercaria, are mostly notocotylids. The present species agrees in the main with Sewell's "Ephemera" group (Faust's "Trioculate" group), with the exception of the excretory system and some variations in the genital system.

The present cercaria shows certain similarities with *C. indicae* XI Sewell (1922), but they differ in certain respects, especially in details of the excretory, digestive, and reproductive systems, and the postero-lateral locomotor organs. Although Sewell's description of the excretory system differs from my description and figures, if one may judge from his drawing, the excretory systems are similar in general form, but not in details. The large, oval excretory bladder, with its greatest length transverse to the axis of the body in Sewell's species, corresponds to the secondary bladder in my species, while the horse-shoe-shaped excretory vessel, filled with excretory concretions, corresponds to the primary bladder and the main lateral trunks in my cercaria. Since Sewell observed the excretory trunks only to the level of the bifurcation of the esophagus, no further comparison between the two species can be made. The intestinal ceca in *C. indicae* XI are S-shaped; in the present species they have a single curve. In both cercariae the reproductive system is well-developed, and some of the organs are similarly placed. The ovary is median in *C. indicae* XI; slightly to the right in my cercaria. In the former, the uterus is slender and straight, and no Mehlis' gland or vitellaria has been observed; in the latter species the uterus is slender and coiled, and both Mehlis' gland and vitellaria are well differentiated.

\* It is apparent that Miss Hsü overlooked the description of *C. infracaudata* by Horsfall (1930) which is obviously much closer to *C. eversum* than any discussed by Miss Hsü. Attention must be called to the following resemblances: time of emergence, encystment, number and time of appearance of eye-spots, excretory system including flame-cell pattern, and reproductive organs. Important differences to be noted are the single excretory tube in the tail of *infracaudata* (two in *eversum*), the position of the vitelline fundaments, and the nervous system. The difference in nervous system may be accounted for by differences in technique. The median eye spot has a lens in *eversum* and none in *infracaudata*. These species are manifestly distinct although congeneric (La Rue).

The postero-lateral locomotor organs also differ. In *C. indicæ XI* the cavity of the locomotor organ contains a pyriform or rounded refractile body which is protruded during locomotion, while in the present cercaria no such structure occurs, but the wall of the cavity can be protruded as previously described. Spines and muscles were not mentioned by Sewell.

Eye-spots and heavy brown or black pigmentation at the anterior end of the body are features common to many monostome cercariae and the descriptions of these structures by Cort (1915), Faust (1917a, 1918, 1919a, and 1922), Dubois (1929), Rees (1932), Wunder (1932), Wesenberg-Lund (1934), and others show that they are similar to those just described in *Cercaria M. eversum*. Although the lines of pigmentation extending posteriorly differ in number with the species, they serve as superficial indices of the underlying nerve trunks as Faust (1917a) has pointed out in *C. pellucida*. Cort (1915) mentioned only two longitudinal dorsal lines of pigment in *C. urbanensis*, while Faust (1917a) in *C. pellucida*, Sewell (1922) in *C. indicæ XI* and others described six pigmented lines, two dorsal, two lateral and two ventral. Rees (1932) and Wesenberg-Lund (1934), however, described four pigmented lines, two dorsal and two ventral, in *C. monostomi*. The findings in the present cercaria agree with those of Cort.

Faust (1918a) in describing *C. pellucida* gave a detailed account of the structures and the innervation of the eye-spots, which are similar to those in *Cercaria M. eversum*. Faust (1922) in *C. plana*, Wunder (1932) in *C. monostomi*, and Wesenberg-Lund (1934) in *C. ephemera* stated that the median eye develops later than the two lateral eyes. The nervous system in *Cercaria M. eversum* agrees in general with that in *C. pellucida* except in respect to the branches given off from the posterior dorsal nerve trunks.

The locomotor organs at the postero-lateral angles of the body are characteristic of monostome cercariae. Looss (1896) described these structures in *C. imbricata* as a large cavity with a cuticular lining, and at its center a pointed projection dividing it into two parts. The pointed projection differs from the out-folding wall of the cavity in the present species. Sinitsin (1905) stated that the locomotor pockets in *C. ephemera* Nitzsch are lined with spines as found by me in this cercaria. Lebour (1907) in *C. ephemera* described these organs as suckerlike structures, circular in outline and divided into two by a bar. Cort (1915) considered the function of the locomotor organs in *C. urbanensis* as analogous to setae, but not to suckers, since no muscles are present. Faust (1917a), however, in *C. pellucida*, *C. konadensis* and *C. urbanensis* Cort, found that the function of these organs is distinctly suctional, and not analogous to setae. He found no spines in his cercariae but observed muscles in *C. pellucida* and glandular cells around the lumen of the invaginated cavity in *C. konadensis*. The muscles and glandular cells in my cercaria agree with Faust's observations, while the spines function like setae. Faust found four muscles attached to



the locomotor organ in *C. pellucida*, while in the present species the organ is provided with circular and longitudinal muscles. Although, as shown above, the locomotor organs have a considerable variation in structure in different species, they always act as a substitute for a ventral sucker in locomotion.

The excretory system seems to differ from that of any previously described monostome cercaria. The characteristic anterior union of the lateral excretory trunks has been described in many monostome cercariae, but the collecting tubules and the arrangement of flame-cells have been completely traced out only in *C. spatula* by Faust (1919), who described the entire excretory system. *Cercaria M. eversum* and *C. spatula* are alike in respect to the general form of their excretory tubes, but their excretory bladders and the flame-cell patterns are different. *C. urbanensis* Cort (1915), *C. pellucida* Faust (1917a), *C. indicae* XI Sewell (1922), and others have a single excretory tubule in the tail, while this cercaria has two. *C. yenchingensis* Faust (1930) has an anterior circuitous tubule with a short blind median pouch extending toward the oral sucker as in my species.

The method of encystment on the protected surface of the operculum of a snail appears not to have been reported for cercariae, but encystment on shells of snail hosts, generally around the aperture, has been reported by Harper (1929) in *Notocotylus seineti* Fuhrmann and by Rees (1932) in *C. monostomi* v. Linst. Wesenberg-Lund (1934) found that *C. ephemera* encysts on the shell of the snail host and that cysts may cover the whole shell.

#### METACERCARIA

Very few monostome life histories have been completed experimentally. In the family *Notocotylidae* are to be listed the studies on *Notocotylus seineti* Fuhrmann by Harper (1929) and *N. attenuatus* Rud. by Joyeux (1922) and by Mathias (1930). Mathias' results, however, differ greatly from those reported by Joyeux.\*

The freed living metacercaria is spatulate and capable of extension and contraction, but in a less degree than the cercaria. Since no growth takes place during the metacercarial stage, there is no essential change in the different structures and organs except that the lateral locomotor organs have disappeared, and there are minor changes in the pigmentation, eye-spots, structure of the wall of the secondary bladder and in the position of the excretory pore. The anterior end is less pigmented, and in the older metacercaria the median eye-spot has disappeared, and the pigmented granules around the lateral eye-spots are more diffuse. The lateral chambers of the secondary bladder are enlarged and their walls have become much

\* To these accounts it is now possible to add Horsfall's observations on the life history of *M. obtusicaudum* (La Rue).

folded giving the appearance in optical section of irregularly-shaped diverticula. Since the tail has been released from the body, the excretory pore has become a terminal, transverse, slit-like opening (fig. 12). Twenty mounted specimens measure 0.432–0.639 mm. by 0.18–0.252 mm. (average 0.558 by 0.217 mm.). The oral sucker measures 0.046–0.074 by 0.05–0.074 mm. (average 0.0637 by 0.065 mm.). In morphology the metacercaria of *M. eversum* varies little from the cercaria. This agrees with what Joyeux (1922) has found in *Notocotylus attenuatus* Rud. Living metacercariae can be obtained only during the summer months, when most cercariae emerge. Metacercariae dissected from cysts during the winter months, with few exceptions, were dead. How long the metacercaria can live within the cyst and remain infective was not determined.

#### DEVELOPMENT OF THE REPRODUCTIVE SYSTEM

Although several species of monostome cercariae with more or less well-developed reproductive systems have been described, the development of their genital organs was not studied. Hence no comparative study is permitted. Reference may be made to work on other kinds of cercariae.

Schwarze (1886) found a solid spherical mass of crowded nuclei which could be distinguished as the genital primordium in the middle of the germ-ball of the stylet cercaria, *C. armata* v. Siebold. This mass later separates into three fundaments, from which eventually develop the organs of the genital system, the anterior fundament giving rise to the cirruspouch, the middle to ovary, uterus, and Mehlis' gland, and the posterior to testes. Both the middle and posterior fundaments remain connected with the anterior fundament by means of strands of cells. Leuckart (1889) found a similar and equally discernible mass of genital cells in the germ-ball of *C. Distomum hepaticum*. This primary fundament is later divided into three groups of cells which develop into various parts of the genitalia as demonstrated by Schwarze. Cary (1909) in his study of the life history of *Diplodiscus temperatus* confirmed all of Schwarze's views concerning the development of genital system, except that he found the first indication of genital primordium occurring in a much later stage of embryonic development.

In developing *Cercaria M. eversum* the genital primordium is first discernible in the posterior half of the germ-ball as a round mass of nuclei, surrounded by a narrow zone of clear homogeneous plasma (fig. 15) similar to what Schwarze (1886) described in *C. armata*. Later this mass becomes separated into indistinct cell-groups whose number is difficult to determine; roughly speaking, however, there are six closely-knit masses (fig. 16). During their later development the general mass elongates and becomes slipper-shaped with two tiny lateral projections from its posterior portion (fig. 17). In this slipper-shaped structure the two anterior cell masses form

the front and middle parts, and the four posterior masses the back part of the two adjoining projections. Sometime later the two tiny projections become partially separated from the main mass to become the fundamentals of testes. These grow laterally and posteriorly while remaining connected with the main fundament by bead-like strands of spindle-shaped nuclei interspersed with homogeneous protoplasm (fig. 18). These strands later become the two vasa efferentia (figs. 19, 20, and 22). Later these ducts can be seen to be united in front of the ovary and connected with the cirruspouch by a strand of spindle-shaped cells, the vas deferens, which runs forward dorsal to the uterus (fig. 21). Whether the testes with their vasa efferentia, the vas deferens and the cirruspouch are all parts of a single male genital primordium or whether the several parts have separate origins which later become united could not be determined. The former condition, however, is suspected.

The two anterior masses gradually draw apart and become clearly outlined fundamentals. The one, club-shaped and lying obliquely, develops into the cirruspouch, and the other, lying laterally and somewhat posteriorly into the metraterm. Meanwhile, posterior to the testicular fundamentals, a pair of cell complexes are given off by the posterior mass; these are the fundamentals of the vitellaria. At the same time the posterior portion of the main mass divides into two groups of cells. Of these the anterior and slightly dextral group is the fundament of the ovary, while the posterior and more median group is the primordium of Mehlis' gland (fig. 18). Gradually the two vitelline cell-complexes grow laterally and anteriorly passing the vasa efferentia (fig. 19). As the developing testes are connected to the main fundament, so the vitelline cell-complexes are connected with the fundament of Mehlis' gland by strands of nuclei which later become vitelline ducts (fig. 20). The vitellaria finally are located anteriorly and ventrally to the two testes (figs. 14, 22, and 29). Thus in the mature cercaria the genital organs are well-developed, as they have been described in several other monostome cercariae.

Discussion: The first indication of the genital primordium agrees very well with that in *C. armata* described by Schwarze (1886), and in *C. Distomum hepaticum* by Leuckart (1889). Schwarze, however, did not closely observe their differentiation into organs and inferred their developmental outcome. Both Schwarze and Leuckart derived the testes from a special fundament which later divides into two, while in my cercaria the testes originate from the two cell-complexes given off by the main mass of cells from which later ovary, Mehlis' gland, and vitellaria arise. It is difficult to determine whether these organs have originated from one or more fundamentals. The strands of spindle-shaped cells connecting the testes with cirruspouch, as described above in the present cercaria, were also mentioned by Schwarze (1886) and Leuckart (1889) in *C. echinata* and *C. Distomum*



*hepaticum* respectively. Looss (1894) gave a detailed description of the same structure in *C. macrocerca*.

Schwarze (1886) derived the vitellaria from parenchyma cells in *Distomum endolobum* and Leuckart (1889) from an independent source in *Distomum lanceolatum*. He stated that they develop in the same manner as the cuticular glands. Looss (1894) demonstrated that in *C. macrocerca* the fundaments of vitellaria originate from the fundament of the female complex just as has been described in my cercaria.

Sinitsin (1931), in his study of the development of the ovary in cercariae and adolecscariae of *Plagioporus*, stated: "The anlage, from which the ovary, albumin gland and Laurer's canal originate, originates independently of the anlage, from which the rest of the genitalia develop. This must be construed as a rule common to all digenetic trematodes." Sinitsin, perhaps, drew a broader conclusion than was warranted. Since his report gives no indication that he had studied the development of the genital system in any stage younger than the mature cercaria, and since the fundaments of all the principal parts of genitalia are well-developed in the mature cercaria, it appears that he did not determine their origin. The fact that the fundament of the ovary appears late in the development of the cercaria, when the other genital organs are already well outlined, is not proof that it originates independently of the fundament. It probably is covered up in the mass of cells and cannot be distinguished until the other organs draw away from it.

#### DEVELOPMENT OF THE EXCRETORY SYSTEM

The development of the excretory system was studied from the germ-ball stage to the adult worm. In the germ-ball stage this system consists of two slightly diverging longitudinal tubes beginning at the middle of the body and extending to the posterior end where each enters a small bladder (fig. 30). As development proceeds these two tubes grow anteriorly, but soon bend laterally and posteriorly, each terminating in a flame-cell. The anterior loop thus formed continues to grow forward and mesiad (fig. 31) with the two parts of each loop close together. The posterior portions of the main excretory trunks also begin to approach each other. Shortly a constriction of the body at the level of these approximating tubes marks about a fourth or fifth portion of the body which develops later into the tail, as first shown by Looss (1892) for *Cercaria Amphistomum subclavatum*. Meanwhile after division of each flame-cell into two, one of the resultant cells with its collecting tubule moves anteriorly along the side of the body and the other posteriorly (fig. 32). The anterior loops of the excretory trunks continue to grow toward each other, finally meet anteriorly and fuse. This is followed by a progressive fusion of the lateral arm of each loop with the trunk contiguous to it, until the point of fusion reaches

nearly half way back along the excretory trunks. Thus of the lateral arm of each original loop there remains only a short branch uniting the collecting tubules of its side with the main excretory trunk (figs. 33 and 34).

During this period of development the four flame-cells divide again, thus giving rise to eight flame-cells, two pairs each, anterior and posterior (fig. 34). Meanwhile, in the region anterior to the junction of the tail and the body, the main excretory trunks continue to draw together until they fuse to form a bladder. From the dorsal surface near the posterior part of the body an invagination penetrates to the bladder with which it forms a connection. Thus a new excretory pore is formed, and at about the same time the two openings at the end of the tail become closed off and now end blindly. Meanwhile each flame-cell of the four pairs gives rise to three, so that there are now twelve flame-cells on each side of the body (fig. 35). This flame-cell pattern, expressed by the formula  $2[(3+3)+(3+3)]$ , remains the same in the mature cercaria (fig. 14).

The development of the bladder and the tail, except the excretory tubes in the tail, much resembles that described by Looss (1892) in *Cercaria Amphistomum subclavatum* and demonstrated by Sinitsin (1911) in a number of developing cercariae. Faust (1919) in developing *Cercaria spatula* indicated the presence of two excretory tubules in the germ-ball stage, the formation of a median bladder by the fusion of the posterior parts of these tubules, and the fusion of their anterior parts to form a tubular circuit, much as these occur in the development of the excretory system of *Cercaria M. eversum*.

Sinitsin (1911) restated the method for the formation of the urinary bladder as first described by Looss, i.e., by the fusion of the main excretory trunks, and then he described a second method, viz. by the proliferation and invagination of epithelial cells to form the lining of the bladder. In the former method, the bladder is mesodermal in origin, while in the latter method, it is derived from the ectoderm. Sinitsin pointed out that the second method is less common than the first.

The present species presents a combination of the two methods mentioned above, since the original bladder has a mesodermal lining which is retained. An excretory pore is formed by invagination on the dorsal side (fig. 23), and shortly afterward the ectodermal cells around the pore proliferate and surround a gradually enlarging cavity which presses into, but is not yet connected with the bladder. These cells carry in a cuticular layer. A narrow clear zone surrounds the proliferating cells. The invaginated part gradually widens and thus forms two chambers which grow laterally and anteriorly, pushing the bladder ahead of them (figs. 24, 25, 26 and 27). The central part of the invagination remains as a vestibule and a median connection is now formed between it and the bladder (fig. 28). Consequently in the mature cercaria the excretory bladder consists of two parts:

the V-shaped bladder of mesodermal origin, hereafter called the *primary* bladder, which receives the main excretory trunks, and the vestibule with its anteriorly directed lateral chambers enclosed in a layer of cells derived from the posterior part of the original primary bladder. The vestibule may now be called the *secondary* bladder.

During the metacercarial stage there is little change in the excretory system, the flame-cell pattern remaining as in the cercaria. However, the walls of the two chambers of the secondary bladder become much folded, giving the appearance in optical section of many short irregularly shaped diverticula, and the excretory pore becomes a terminal transverse slit (fig. 12).

In the adult the form of the excretory tubes remains the same as in the metacercaria, but the flame-cell pattern is undiscernible because of the opacity of the tissues. There is a further increase in the number of folds in the walls of lateral chambers of the secondary bladder, giving the appearance of many diverticula in the lumina of these chambers. It is notable that the secondary bladder and its lateral chambers are at times thrust out through the slit-like transverse excretory pore, so that they resemble two ambulacral feet of an echinoderm (fig. 8). The tissues surrounding the excretory pore are very muscular, and the pore itself is capable of great distension. When the two tubes are fully extended, the posterior part of the body is rolled back in a thick roll. This heavy musculature controls the opening and closing of the external excretory pore. Mackin, who apparently worked on preserved material, reported no such movements of the vestibule and the diverticula.<sup>1</sup>

Mackin (1930) described for *Macravestibulum obtusicaudum* an excretory system very similar to that described above, except for the apparent lack of an opening between primary excretory bladder and vestibular cavity in his species.<sup>2</sup>

Walter (1893) in describing the urinary bladder of *Monostomum trigonocephalum* mentioned the presence of elongated ribs furnished with ciliated epithelium. These structures were later described by Looss (1902) who called the bladder with these ribs "Rippentrichter." Looss found this structure in *Pronocephalus obliquus*, *Pleurogonius bilobus*, *P. longiusculus*,

<sup>1</sup> Horsfall (1935) recorded her observations in these words: "The immature worm fastened itself to the substratum by its sucker and concave ventral surface, evaginated the vestibular cavity, and slowly waved the posterior end of the body back and forth (fig. 18). The body which was encircled by folds or creases due to the vestibular evagination bore a superficial resemblance to certain members of the Hemiuridae. Any sudden change caused invagination of the vestibule and the worm assumed its more typical appearance. This reaction lasted for only a few hours after removal from the host. The author could not attach any significance to this behavior, and sectioned specimens gave no clue to its cause."

<sup>2</sup> Horsfall (1935), however, in the same species "was able to demonstrate 3 to 6 tiny ducts leading posteriad from the most posterior median part of the bladder to the vestibule."



*Glyphicephalus solidus*, *G. lobatus* and *Epibathra crassa*, all from marine turtles. It consists of elongated ribs projecting from the inner surface of the excretory bladder. The surface of these ribs is provided with fine bristles which give the appearance of heavy cilia. From his figures one must judge that perhaps a part of the inner surface of the bladder is cuticular in structure and hence ectodermal in origin. Mackin (1930) compared the vestibular cavity of *M. obtusicaudum* with the vestibule in Looss's species thus: "The enormous development of the vestibular cavity is so striking and unique, that it has no parallel in the family. There can hardly be any doubt that the structure is homologous to Looss's 'Rippentrichter,' although the general form of the organ is at great variance with any of the forms he described."

Walter (1893), in *Monostomum proteus* and *M. reticulare*, described rosette-formed diverticula as out-pockets from the excretory bladder lined with indistinct ciliated epithelium. Looss (1902) also mentioned these rosette-formed diverticula in his description of the urinary bladder of the monostome species, *Microscaphidium reticulare*, *M. aberrans*, *Octangium sagitta*, *O. hasta*, and *Deuteroberis proteus* found in marine turtles. The number of these diverticula varies with the species but they are arranged in the same radiating fashion. The inner surface of the diverticula is lined with ciliated epithelium, but their basal part is covered with cuticle which is a continuation of the cuticula of the body. Apparently in these species the cuticular part of the bladder is formed by the ingrowth of the ectoderm surrounding the pore, and is thus ectodermal in origin. In spite of the fact that these species belong to the family *Angiodictyidae*, these organs present certain resemblances to the vestibule of *Macravestibulum* and a further comparison of these organs should here be made. In *M. eversum* the lateral chambers, together with their folded diverticula are also out-pockets of the secondary bladder though they are not arranged in a rosette around the excretory pore. Furthermore, the lining of its secondary bladder is cuticular, while in the species described by Looss the cuticular lining is confined to the basal region of the diverticula. It is evident, therefore, that the structure of the secondary bladder of *Macravestibulum* not only resembles, but actually is homologous to, the rosette-shaped diverticula in the bladders of the species described by Looss.

#### SUMMARY

The life history of a new species of *Macravestibulum* has been completed experimentally from cercaria to adult, the second to be worked out in the Family *Pronocephalidae*. The morphology of the respective stages in the life history has been described. The development of the reproductive system and the locomotor organs of the cercaria has been traced from the germ-ball to the mature cercarial stage, while the development of the ex-

cretory system has been studied from the germ-ball to the adult stage.

1. The definitive host is the map-turtle, *Graptemys geographica* (Le Sueur); the snail host, *Goniobasis livescens* (Menke).
2. Encystment of the cercaria takes place on the protected surface of the operculum of the snail host.
3. The locomotor organs at the postero-lateral angles of the body of the cercaria are formed by invagination of ectodermal tissues carrying with them the cuticula.
4. The genital primordium is first discernible in the germ-ball of the cercaria as a rounded mass of nuclei from which develop the different parts of the genital organs which, in the mature cercaria, are well-developed.
5. The excretory system in the germ-ball stage of the cercaria consists of two longitudinal tubes. These are converted during development of the cercaria into tubules and twelve pairs of flame-cells, two lateral trunks joined anteriorly, and the primary excretory bladder. The excretory pore and a secondary bladder consisting of a vestibule and two eversible lateral diverticula result from an invagination from the dorsal surface.
6. In the metacercarial and adult stages the excretory pore becomes a terminal, transverse opening, and the lateral diverticula of the secondary bladder become enlarged with their walls much folded.

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